Cell proliferation and ultrastructure in the roots of young wheat seedlings induced by oxidative stress

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Desiccation stress in plants is often accompanied by universal stress responses such as the accumulation of oxygen radicals resulting in oxidative stress. This in turn may lead to the depression of the early development of seedlings after the germination of orthodox seeds. To study the negative effects of oxidative stress on the growth of plant cells, we investigated the production of reactive oxygen species (ROS), the changes in mitotic index, growth and cell ultrastructure induced by the classical pro-oxidant paraquat (methylviologen). We also tested the effects of salicylic acid (SA), a signal molecule involved in plant stress tolerance and a growth regulator but that can also facilitate an increase in ROS and down regulate the cell cycle and cell proliferation. Both SA and paraquat at concentrations of 0.01–1.0 mM caused the accumulation of superoxide radicals and H_2O_2, decreased cell mitotic index and inhibited root growth in 5 day old wheat (*Triticum aestivum* L.) seedlings. These effects were accompanied by the changes in cell ultrastructure. Treatment of roots with 1 mM SA for 6 h caused degradation of the membrane lipids and an increase in the number of peroxisomes. Paraquat (0.001–0.10 mM) induced the accumulation of myelin-like bodies, reduced the electron density of the mitochondrial matrix, and caused collapse of the tonoplast. A common characteristic of both SA and paraquat induced effects on cell ultrastructure was the appearance of numerous autolytic vacuoles containing mitochondria, cytoplasm and other organelles. Thus, the accumulation of ROS can result in the depression of plant growth and cell proliferation and further, can induce autophagy like processes.

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A protocol for the production of adventitious shoot explants for future cryopreservation of *Ekebergia capensis* using a temporary immersion system (RITA®)

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Ekebergia capensis Sparrm. is an indigenous African species that produces unstorable, recalcitrant seeds. This poses major problems for the long–term storage of such germplasm. Work with excised axes has revealed that the shoot apical meristem sustains lethal damage consequent upon excision from the cotyledons, although adventitious buds can be induced to form at the wound sites. The phenomenon of shoot apex necrosis, which seems to be common when axes of tropical species are excised (see abstract by Goveia et al.), prompted our research group to investigate the potential of alternative explants for cryopreservation of the genetic resources of recalcitrant-seeded species. The present contribution reports the preliminary findings on the development of a protocol for the production of adventitious shoots from intact root explants of *Ekebergia capensis*. Roots produced by *in vitro* germination of excised embryonic axes, were placed in a temporary immersion bioreactor (RITA®) containing liquid media supplemented with the cytokinin, BAP. After 24 h, the roots were plated on standard semi-solid germination medium. After 4 weeks, adventitious shoots were produced from the root explants. This protocol was performed using seeds obtained from two locations *viz*. Port Elizabeth (33°30′ S) and Mtunzini (28°22′ S), and a difference in the response of germplasm from these two locations was observed. (For further provenance-related differences in the responses of seeds of *E. capensis*, see the Abstract and poster of Bharuth et al.) The adventitious shoots produced in this manner were cultured on elongation medium for further growth and development. Once shoots were sufficiently large, they were rooted on medium supplemented with no auxin or only a low IBA level. This work has important implications for the storage of species that produce recalcitrant seeds as it provides a potential strategy for the production of an alternate source of explants that can be used for the cryopreservation of germplasm.

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Assessment of a strategy to curtail fungal proliferation in non-orthodox seeds during storage

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Recalcitrant seeds usually host a range of active bacterial and fungal contaminants at harvest. The storage conditions necessary to maintain viability of intact tropical recalcitrant seeds are high humidity and relatively high temperatures which are also conducive to fungal and bacterial proliferation. A number of deteriorative changes accompany the presence of fungi during storage of recalcitrant seeds, and the hydrated storage lifespan, particularly of seeds of tropical species can be considerably extended if fungal activity is curtailed. Simple surface sterilization is effective only in removing contaminants on the seed surfaces, thus to implement successful long-term storage and axis cryostorage, elimination of intraseminal fungi is imperative. In this study the use of systemic fungicides applied as a preliminary soak before hydrated storage was evaluated as one method of removing internal fungal contaminants from the recalcitrant seeds of the tropical species *Trichilia dregeana*. Three different fungicides namely: Orius 200 EW® (active ingredient tebuconazole [200 g l^{-1}]); Heritage®, a systemic translaminar fungicide (active ingredient azoxystrobin [0.5 g g^{-1}]); and Celest 100FS® (active ingredient phenylpyrole [100 g l^{-1}]) were